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for Prostate Cancer

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The goal of this proposal is to evaluate prostatic acid phosphatase (PAP), a prostate tissue-specific protein, as a candidate tumor antigen for a prostate cancer vaccine. PAP is a well-defined protein, whose expression is limited to normal and malignant prostate tissue. In addition, data presented suggests that some patients with prostate cancer have a pre-existent immune response to PAP, suggesting that immune tolerance to this "self" protein can be circumvented. Studies described here will evaluate the immune response to PAP in patients with various stages of prostate cancer, characterize the T helper subset active in endogenous PAP-specific immunity, and test whether PAP-specific CTL derived from individual patients can lyse prostate tumor cells. The results of investigations described in this proposal will lead to a human phase I clinical vaccine trial targeting PAP in patients with prostate cancer. The specific aims of the current proposal are: (1) to determine whether patients with prostate cancer have a pre-existing CD4+ T cell immunity to PAP, and (2) to determine whether patients with prostate cancer have a pre-existing CD8+ T cell response to PAP and whether PAP-specific CTL derived from individual patients can lyse prostate tumor cells.

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Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	5
Key Research Accomplishments.....	8
Reportable Outcomes.....	9
Conclusions.....	N/A
References.....	N/A
Appendices.....	10

First year Review for Proposal: *Evaluation of Prostatic Acid Phosphatase (PAP) as a Candidate Antigen for the Development of Cancer Vaccines for Prostate Cancer.*
(Funding period 10/99 to present)

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INTRODUCTION: The goal of this research is to evaluate prostatic acid phosphatase (PAP), a prostate tissue-specific protein, as a candidate tumor antigen for a prostate cancer vaccine. As stated in the original proposal and statement of work, the specific aims of the work are to: (1) determine whether patients with prostate cancer have a pre-existing CD4+ T cell immunity to PAP, and (2) determine whether patients with prostate cancer have a pre-existing CD8+ T cell responses to PAP and whether PAP-specific CTL derived from individual patients can lyse prostate tumor cells. This report will review the accomplishments made over the first year of funding with respect to the specific aims and statement of work of the project.

Aim 1: To determine whether patients with prostate cancer have pre-existing CD4+ T cell immunity to PAP.

□ Purification of PAP from CHO cells – TASK FINISHED

During the first three months of work I was unsuccessful in generating sufficient quantities of purified recombinant human PAP from CHO cells to perform the T cell work proposed. Therefore, the studies below were initiated and completed using highly purified human PAP available through commercial vendors (Research Diagnostics, Inc. and Chemicon International).

□ Collection of blood and proliferative T cell assays from 120 patients and 30 normal patients – TASK FINISHED

Due to the cost of reagents and available cryopreserved peripheral blood mononuclear cells from patients with prostate cancer, the initial scope of this task was scaled down to an analysis of 80 patients with varying stages of disease (20 patients per group as originally described) and 20 volunteer control donors. The work was extended, however, to also look at T cell proliferative responses to PSA in the same patient panel. The completed analysis demonstrated that 14% (11/80) of patients have significant (stimulation index > 2) CD4+ T helper cell responses to PAP. This is not statistically different from the control population, in which 5% (1/20) controls also had a detectable T cell response to PAP, and no differences were noted among the subpopulations of patients. These results were in contrast to those found for PSA, in which 7.5% of patients had significant responses to PSA compared with none of the controls. Responses were also more common in patients with metastatic disease (15%, 6/40) compared with early stage disease (0%, 0/40). These results were presented in a poster presentation at the April 2000 national meeting of the American Association for Cancer Research.

□ Characterization of cytokine profile for PAP-specific helper T cell responses – TASK FINISHED

The CD4+ helper T cell responses to both PAP and PSA above were characterized by cytokine release and shown to be predominantly IFN γ -secreting as opposed to IL-5-secreting, consistent with a Th1-like response. Taken together, these results have been significant to demonstrate that patients with prostate cancer do indeed have low-level T cell responses to PAP, and the type of T cell response is consistent with a potentially therapeutic response. These results were presented in the same poster presentation as above at the April 2000 national meeting of the American Association for Cancer Research.

□ Characterization of antibody responses to PAP in 120 patients and 100 normal controls – TASK FINISHED

This analysis was extended to a larger patient population (200 patients) and to a larger analysis of other prostate cancer-associated proteins. Antibody responses were consequently analyzed by ELISA to PAP, PSA, p53 and HER-2/neu in the same population of 200 patients. Only a minority of patients (6%, 12/200) had a detectable antibody titer to PAP, and this was not statistically distinct from the control population (7%, 7/100, $p=0.74$). Similar results

were found for p53. Antibody responses were significantly different in the patient populations for PSA (11%, 22/200) and HER-2/neu (15.5%, 31/200) compared with controls (3%, 3/100, $p=0.02$, and 2%, 2/100, $p=0.0004$ respectively). These responses were most prevalent in the subpopulation of patients with androgen-independent prostate cancer. These results have suggested that the primary immune response to PAP is cellular, and Th1-biased, not antibody-biased. This antibody study in particular, however, has demonstrated that patients with prostate cancer, and even late-stage patients, are able to mount an antigen-specific immune response to proteins made by their tumors. These results have been submitted and accepted for publication in the *Journal of Urology*.

□ Characterization of T helper cell peptide epitopes recognized – TASK INITIATED AND ONGOING

The immunogenicity of PAP-specific T helper cell epitopes has been evaluated in 20 patients with prostate cancer. While the initial proposal was to evaluate 40 patients overall, 20 patients with proliferative responses to PAP, and 20 without, this was not feasible given the low frequency of responses found above and the availability of cryopreserved specimens for the analysis. Consequently, T cell responses to individual peptides have been analyzed in 7 patients with T cell responses to PAP and 13 patients without T cell responses to PAP. These studies identified 4 peptides that are likely CD4+ T cell epitopes, in that they were recognized only in patients with T cell responses to PAP. These results were presented at a poster presentation at the American Association for Cancer Research annual meeting in April 2000. I am currently in the process of trying to expand T cell lines *in vitro* from PBMC obtained from patients without T cell responses to PAP using these peptides as stimulator antigens. The goal of these studies will be to determine if these peptides may be useful for generating T cell responses to PAP, and therefore potentially useful for inclusion in vaccines targeting PAP in human trials. To date, short-term lines have been established on 3 of the 4 peptides which demonstrate peptide-specific proliferation. Two of these have also demonstrated PAP-specific proliferation. Work ongoing will validate these results and T cell clones will be established for finer specificity testing and to elucidate the pattern of cytokine secretion.

Aim 2: To determine whether patients with prostate cancer have a pre-existing CD8+ T cell responses to PAP and whether PAP-specific CTL derived from individual patients can lyse prostate tumor cells.

□ Generation of target cell lines from subject fibroblasts and B-LCL – TASK INITIATED AND ONGOING

To date, we have established fibroblast lines and EBV-transformed B cell lines in the context of other funded clinical studies in 14 HLA-A2 expressing patients with prostate cancer. These cell lines will be used as stimulator and target cell lines for the experiments proposed. The cDNA for human PAP has been cloned into a pCDNA expression vector as well as a

retroviral vector (pLNCX). We are in the process of transforming these cell lines with these constructs. The tasks below will be initiated when target cell lines are available for analysis.

- Determination of PAP-specific CTL frequency by limiting dilution in a chromium release assay for 10 normals and 20 patients with prostate cancer – TASK NOT YET INITIATED
- Determination of PAP-specific CTL frequency by ELISPOT for 10 normals and 20 patients with prostate cancer – TASK NOT YET INITIATED
- Define HLA-A2 epitopes able to generate PAP-specific CTL using patient PBMC *in vitro* – TASK INITIATED

The eleven potential HLA-A2 binding peptide epitopes from PAP have been constructed and, in a project not identified in the proposal, have been ranked in an *in vitro* T2 binding assay for their true binding affinity for HLA-A2. These results have identified 6 peptides with moderate-to-high binding affinity to HLA-A2. These peptides have then been used as stimulator antigens to generate peptide-specific T cell lines from PBMC obtained from ten HLA-A2 expressing patients. After multiple *in vitro* stimulations I have been unable to detect reproducible peptide-specific CTL activity as determined by chromium release assay using peptide-loaded target cells. This approach has been put on hold while I optimize the system using two other well-characterized A2-binding epitopes from foreign antigens.

- Define HLA-A2 epitopes able to generate PAP-specific CTL using HLA-A2 transgenic mouse model *in vivo* system – TASK INITIATED AND ONGOING

The peptides constructed will also be tested *in vivo* in the HLA-A2 transgenic mice model proposed, with the goal of determining whether these peptides can elicit CD8 T cell responses *in vivo*. Pilot experiments revealed that simple immunization with a boost 2 weeks later were not sufficient to initiate a peptide-specific T cell response. Consequently, prior to definitive testing, this system is being optimized using a well-characterized HLA-A2 binding peptide (influenza matrix peptide) to determine the optimal immunization schedule and assay technique (ELISPOT versus chromium release assay versus intracellular cytokine staining using unstimulated splenocytes), as well as the need for co-immunization with a helper peptide epitope as described by other groups. Once this system is optimized, the mice will be immunized in similar fashion with the eleven constructed peptides. Results from these studies are, obviously, still pending.

- Determine whether T cell lines and clones specific for PAP-derived CTL peptides can lyse autologous cells expressing PAP and/or HLA-matched prostate tumor cell lines – TASK NOT YET INITIATED

Key Research Accomplishments in First Year of Funding:

- ❑ Identification of helper T cell responses, predominantly of Th1 type, in patients with prostate cancer specific for PAP. This suggests that a potentially therapeutic immune response to PAP can exist *in vivo* and therefore may either be initiated or augmented by means of antigen-specific vaccination.
- ❑ Evaluation of humoral immune responses to several prostate cancer-associated antigens has demonstrated that antibody responses to PAP are rare compared with PSA or HER-2/neu, and that the majority of pre-existent immune responses to PAP are cellular responses.
- ❑ Identification of 2 potential CD4 T cell epitopes that may be useful in clinical trials to elicit helper T cell responses to PAP.
- ❑ Identification of 6 peptides derived from the amino acid sequence of PAP which exhibit moderate-to-high binding to HLA-A2 in a T2 *in vitro* binding assay.

Reportable Outcomes in First Year of Funding:

- ❑ Manuscripts, abstracts and presentations:
 - ❑ McNeel DG, Nguyen LD, Storer BE, Vessella R, Lange PH, Disis ML. "Antibody immunity to prostate cancer associated antigens can be detected in the serum of patients with prostate cancer." *J. Urol.* (in press, 2000).
 - ❑ McNeel DG, Nguyen LD, Disis ML. "Identification of potential CD4+ T helper epitopes derived from the protein sequence of prostatic acid phosphatase (PAP)." *Proc. Amer. Assn. Cancer Res.* 41:699 (2000).
 - ❑ McNeel DG, Nguyen LD, Disis ML. "T cells derived from patients with prostate cancer secrete IFNg in response to stimulation with prostate cancer antigens." *Proc. Amer. Assn. Cancer Res.* 41:880 (2000).
- ❑ Patents and licenses – NONE
- ❑ Degrees obtained – NONE
- ❑ Development of cell lines, tissue or serum repositories

Cryopreserved fibroblast lines and peripheral blood mononuclear cells obtained from other clinical studies have been used to establish autologous systems with fibroblast lines and EBV-transformed B cell lines from individual patients. Work is ongoing to establish PAP-expressing fibroblast lines and B-LCL lines. Likewise, archived serum samples obtained from other clinical studies was used to accomplish the work outlined in Aim 1 above.
- ❑ Informatics – NONE
- ❑ Funding applied for based on work supported by this award – NONE
- ❑ Employment or research opportunities applied for - NONE

ANTIBODY IMMUNITY TO PROSTATE CANCER ASSOCIATED ANTIGENS CAN BE DETECTED IN THE SERUM OF PATIENTS WITH PROSTATE CANCER

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ABSTRACT

Purpose: Several immune based therapies targeting prostate cancer associated proteins are currently undergoing clinical investigation. In general, however, little is known about the immunogenicity of prostate cancer or which prostate cancer associated proteins elicit immune responses. We determine whether patients with prostate cancer have antibody immunity to known prostate cancer associated proteins, what the prevalence of this immunity is and whether immunity to individual proteins is associated with the stage of disease.

Materials and Methods: We evaluated the inherent humoral immune response against prostate specific antigen (PSA), prostatic acid phosphatase, p53 and HER-2/neu, all known prostate cancer associated proteins, in 200 patients with various stages of disease and male controls.

Results: Antibody immunity to PSA was significantly different between the patient (11%, 22 of 200) and control populations (1.5%, 3 of 100, $p = 0.02$), and titers 1:100 or greater were particularly prevalent in the subgroup of patients with androgen independent disease (11%, 6 of 56). Antibody immunity to prostatic acid phosphatase and p53 was detected (5.5%, 11 of 200 and 6%, 12 of 200), and was not different from the control population (4%, 4 of 100, $p = 0.57$ and 7%, 7 of 100, $p = 0.74$). Antibody immunity to HER-2/neu was significantly higher in patients with prostate cancer (15.5%, 31 of 200) compared to controls (2%, 2 of 100, $p = 0.0004$), and titers 1:100 or greater were most prevalent in the subgroup of patients with androgen independent disease (16%, 9 of 56).

Conclusions: These findings suggest that prostate cancer is an immunogenic tumor. Moreover, for PSA and HER-2/neu the prevalence of antibody immunity was higher in patients with androgen independent disease, indicating that even patients with advanced stage prostate cancer can have an immune response to their tumor.

KEY WORDS: antibodies, immunity, prostatic neoplasms, prostate-specific antigen

Several prostate cancer antigens are under clinical investigation as potential targets for immune based treatments of prostate cancer, including prostate specific antigen (PSA)^{1,2} and prostatic acid phosphatase (PAP).³ Despite the fact that clinical prostate cancer vaccine trials are under way, little is known about the inherent immunogenicity of prostate cancer. In fact, it has been suggested that the prostate gland is immunologically favored, given the purported immunosuppressive characteristics of the seminal fluid⁴ and the supposed absence of a lymphatic system within the prostate.⁵ Moreover, research performed nearly 20 years ago suggests that cell mediated immune responses are depressed in patients with prostate cancer, as judged by lymphocyte proliferation in response to mitogens, delayed type of hypersensitivity skin testing to dinitrochlorobenzene and T cell aggregation studies.^{6,7} Later studies implicate the loss of MHC class I expression as a mechanism of immune escape by prostate cancer cells.^{8,9}

Within the last 20 years several proteins have been identified whose expression is essentially limited to the prostate, including PSA and PAP, and several other proteins have

been identified likely involved in the metastatic progression of prostate cancer. Unlike melanoma, however, in which tumor antigens have been identified based on their inherent immunogenicity,¹⁰⁻¹² to our knowledge there have been no reports on the immunogenicity of these prostate cancer associated proteins.

We address the question of the inherent immunogenicity of prostate cancer by looking at antibody immunity to PSA, PAP, p53 and HER-2/neu, which are prostate cancer associated proteins that are known tumor antigens. PSA and PAP were chosen as the 2 best characterized prostate specific proteins. There has been a report of a probable humoral immune response to PSA occurring in patients with metastatic prostate cancer,¹³ and others have demonstrated that it is possible to culture human cytotoxic T lymphocytes in vitro specific for PSA^{14,15} and PAP,¹⁶ suggesting that tolerance to these proteins might be circumvented in vivo. p53 and HER-2/neu were chosen as biologically relevant proteins, each implicated in the metastatic progression of prostate cancer and each previously shown to be tumor antigens in breast cancer.^{17,18}

In our study we detected an antibody response to 1 or more of these 4 proteins in 37% (74 of 200) of patients compared with 16% (16 of 100) of male controls. Antibody responses to PAP (5.5%, 11 of 200) and p53 (6%, 12 of 200) were not significantly different from the control populations but antibody responses to PSA (11%, 22 of 200) and HER-2/neu

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(15.5%, 31 of 200) were significantly different from the control populations ($p = 0.02$ and $p = 0.0004$, respectively). Higher titer responses were most prevalent in patients with androgen independent prostate cancer, although this did not reach statistical significance for PSA. These findings indicate that prostate cancer is an immunogenic tumor, and that even patients with advanced stage prostate cancer can mount an antibody immune response to proteins expressed by the tumor.

METHODS

Patient populations. With informed consent, sera were obtained from 200 patients with prostate cancer at the University of Washington Medical Center between 1997 and 1999. Sera were grouped according to disease stage and treatment status. Pretreatment sera were drawn within 1 week of surgery from 48 patients undergoing radical prostatectomy (group 1). Group 2 consisted of 55 patients with stage B or C tumors, who had been treated with radical prostatectomy or brachytherapy and had no evidence of disease recurrence. These sera were collected at a variety of times following surgery. Group 3 included 41 patients with stage D prostate cancer, on androgen ablative therapy and with evidence of androgen responsive disease by PSA monitoring. Group 4 consisted of 56 patients with metastatic, androgen independent prostate cancer. Sera in these latter 2 groups were drawn at different times in the treatment course of individual subjects. Control sera were obtained from male volunteer blood donors 37 to 73 years old, without histories of prostate or other cancers, who contributed blood products at the Puget Sound Blood Bank. All sera were stored in aliquots at -20°C until used.

Detection of PAP and PSA specific antibody responses in patients with prostate cancer by enzyme-linked immunosorbent assay (ELISA). Antibodies recognizing PAP and PSA were detected by ELISA using highly purified proteins. We added $2\text{ }\mu\text{g/ml}$ purified protein in 50 mM sodium carbonate buffer ($\text{pH } 9.6$) to experimental wells overnight at 4°C . Plates were then blocked with phosphate buffered saline/1% bovine serum albumin for 1 hour at room temperature. After washing with phosphate buffered saline/0.1% Tween-20, plates were then probed with human sera at concentrations of 1:25, 1:50, 1:100 and 1:200 for 1 hour at room temperature, with serum added to experimental and blank wells. Serum from a patient with previously documented antibodies to PAP and PSA was included on each plate as a positive control and to serve as an interplate control. After a 1-hour incubation plates were again washed and a peroxidase-conjugated sheep anti-human Ig antibody diluted 1:5,000 in phosphate buffered saline/1% bovine serum albumin was added. Following a 45-minute incubation at room temperature, the plates were washed and developed with tetramethylbenzidine peroxidase substrate according to manufacturer instructions.

Reactions were monitored at optical density₆₅₀ and stopped with addition of hydrochloric acid to 0.5 N concentration when the optical density of the positive control sera reached 0.3. The plates were then read at optical density₄₅₀ and the change in optical density was determined as the difference between the optical density of experimental and blank wells. Titers were calculated using a cutoff value determined from the mean ± 2 standard deviations (SD) of the control population change in optical density values at the most concentrated sera dilution used (1:25). The change in optical density of the positive control sera varied by less than 1 SD among plates, and titered at 1:200 for PSA and 1:400 for PAP. All titers greater than 1:50 were confirmed in an independent assay.

Detection of p53 specific antibody responses in patients with prostate cancer by ELISA. Antibodies recognizing nonmutated p53 were detected using a capture ELISA method,

similar to that previously reported.¹⁹ Briefly, 96-well Immulon 4 plates were incubated overnight at 4°C with a mouse monoclonal antibody specific for human p53 in 50 mM sodium carbonate buffer ($\text{pH } 9.6$). Plates were then blocked as described previously, and a lysate from a p53 abundant human breast cancer cell line, BT20, was added to experimental wells as a source of antigen. After incubation, plates were then washed, probed with human sera at concentrations of 1:25, 1:50, 1:100 and 1:200, and developed as described previously. Serum from a single patient with previously documented antibodies to p53 was included on each plate as a positive, interplate control. Titers were calculated as described previously.

Detection of HER-2/neu-specific antibody responses in patients with prostate cancer by ELISA. Capture ELISA was performed as previously reported,¹⁸ using an IgG specific secondary antibody. As for the other ELISA assays, serum from a single patient with a previously documented IgG antibody response to HER-2/neu was included on each plate as a positive control and to serve as an interplate control.¹⁸ Likewise, all titers greater than 1:50 were confirmed in an independent assay. Previous studies have shown that antibody responses with titers 1:100 or greater are not found in noncancer control sera.¹⁸ Statistical comparison of study populations was performed using a chi-square test, with $p \leq 0.05$ considered statistically significant.

RESULTS

Antibody immunity to PSA can be detected in the serum of patients with prostate cancer. Sera from 200 patients with various stages of prostate cancer and from 100 volunteer male control blood donors were screened for the presence of antibodies to PSA as described (fig. 1). Detectable titers (1:50 or greater) were found in 22 (11%) of 200 patients, which was statistically different from the control population (3%, 3 of 100, $p = 0.02$). The difference between patients and controls was more striking at titers 1:100 or greater, as 6.5% (13 of 200) of patients had detectable antibodies compared with none of the controls (0 of 100, $p = 0.009$). Analysis by stage of disease also suggested that antibody responses were most common in patients with androgen independent disease (6 of 56 with titers 1:100 or greater, 10.7%). However, this subgroup of patients was not statistically different from the combined other subgroups of patients ($p = 0.13$). No correla-

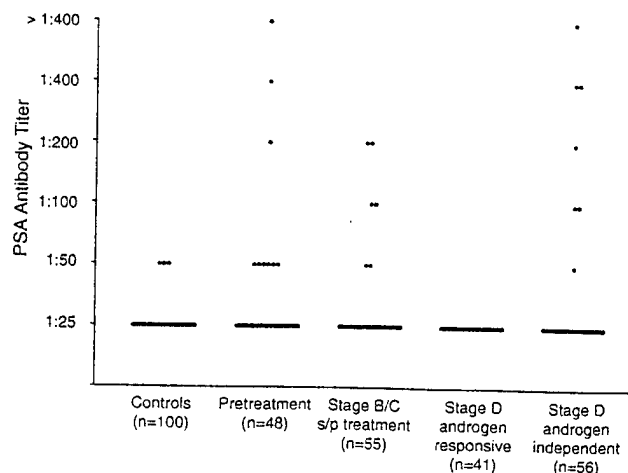


FIG. 1. Antibody immunity to PSA can be detected in serum of patients with prostate cancer. Sera from 200 patients with various stages of prostate cancer and 100 volunteer male blood donors were screened for presence of antibodies to PSA by ELISA. Individual dots represent titers determined for all individual patients or controls. s/p, status post.

tion was noted between the presence of antibodies and serum level of PSA (data not shown). Most of the antibody responses detected were of low titer (1:50 to 1:200) but a few patients had high titer antibody responses (fig. 2). In addition, 2 patients with detectable antibodies to PSA also had detectable antibodies to either PAP or HER-2/neu.

Patients with prostate cancer have antibodies to PAP. Of the 200 patients 11 (5.5%) had antibody immunity to PAP (fig. 3), which was not different from the control population (4 of 100, 4%, $p = 0.57$). No differences were noted with respect to stage of disease. Of interest, however, the patient with the highest antibody titer to PAP also had detectable antibodies to PSA.

Patients with prostate cancer have antibodies to p53. Of the 200 patients 12 (6%) and of the 100 controls 7 (7%) had low titer antibody immunity to p53 (fig. 4). The patient population was not statistically different from the control population ($p = 0.74$). No differences were noted with respect to disease stage.

Antibody immunity to HER-2/neu can be detected in the serum of patients with prostate cancer and is most prevalent in patients with androgen independent disease. Sera from the 200 patients with prostate cancer were screened for antibodies to HER-2/neu using the titer based assay described previously,¹⁸ and the results were compared with the 100 controls. Overall, 31 (15.5%) of 200 patients had detectable antibodies to HER-2/neu compared with 2 (2%) of 100 controls ($p = 0.0004$) at titers 1:50 or greater (fig. 5). At titers 1:100 or greater, at which it has previously been reported that antibody responses are not detected in patients without cancer,¹⁸ 8.5% (17 of 200) of the patient population had detectable antibodies ($p = 0.003$). At this titer HER-2/neu-specific antibody responses were most prevalent in patients with androgen independent disease (9 of 56, 16%) compared with the other combined subgroups of patients (8 of 144, 5.6%, $p = 0.02$). As indicated previously, 1 patient with advanced stage disease and antibodies to HER-2/neu also had detectable antibodies to PSA.

DISCUSSION

There is currently much enthusiasm about the use of immune based therapies for the treatment of prostate cancer. Unlike melanoma, however, in which tumor associated anti-

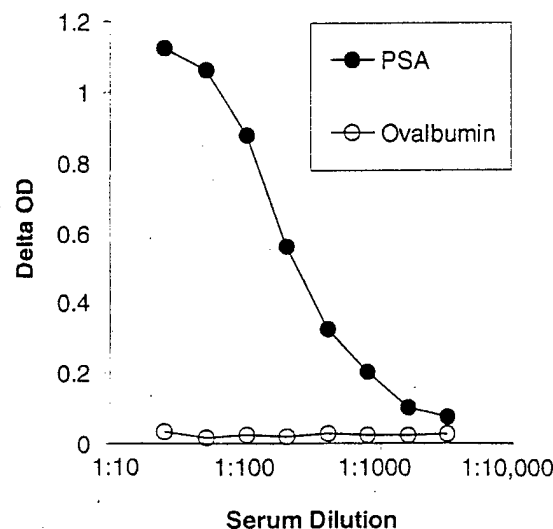


FIG. 2. Some patients have high titer antibody responses to PSA. Results of ELISA detecting antibodies to either PSA or ovalbumin (negative control) in patient with metastatic androgen independent prostate cancer. PSA antibodies titrated at 1:1,600. OD, optical density.

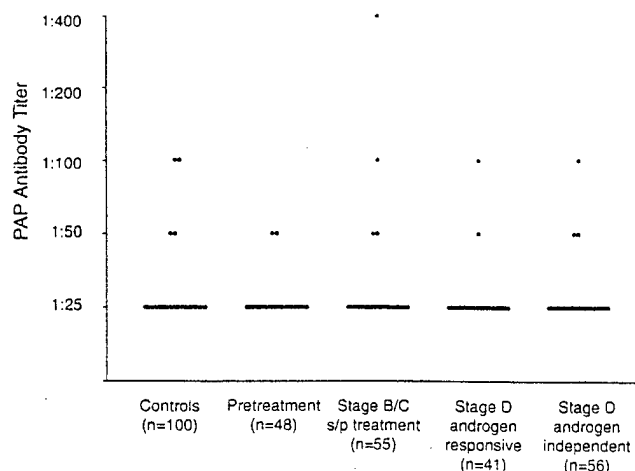


FIG. 3. Patients prostate cancer have antibodies to PAP. Sera from 200 patients with various stages of prostate cancer and 100 volunteer male blood donors were screened for presence of antibodies to PAP. Dots represent titers determined for all individual patients or controls. s/p, status post.

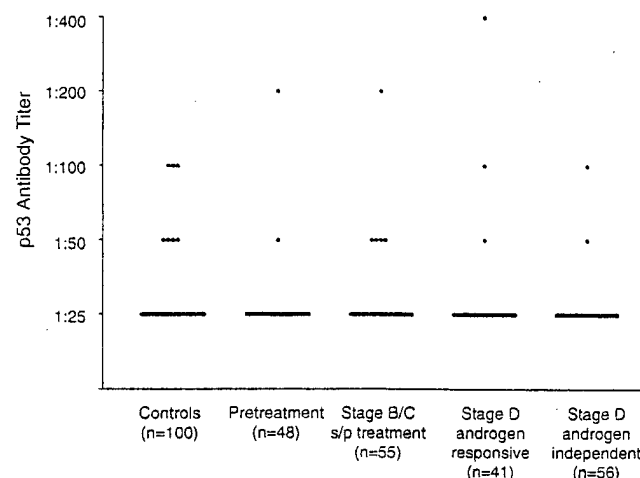


FIG. 4. Patients with prostate cancer have antibodies to p53. Sera from 200 patients with various stages of prostate cancer and 100 volunteer male blood donors were screened for presence of antibodies to p53. Dots represent titers determined for individual patients or controls. s/p, status post.

gens have been defined based on the endogenous immune response to particular proteins,^{11,20} there has been little investigation of the immunogenicity of prostate cancer associated proteins. We report an overview of the humoral immunogenicity of 4 prostate cancer associated proteins, 2 of which are essentially prostate specific, and 2 of which are biologically relevant to the progression of prostate cancer and have been shown to elicit immune responses in patients with other tumors. The specific questions we addressed were do patients with prostate cancer have antibody immunity to known prostate cancer associated antigens, what is the prevalence of this immunity and, is immunity to individual proteins associated with the stage of disease.

Patients with prostate cancer can have antibody immunity to prostate cancer associated proteins. While there were no cancer specific humoral responses detected to PAP or p53, antibody responses to PSA and HER-2/neu were significantly different from a control population. It is unclear why there would be more antibody responses detected to PSA than PAP, given that they are both prostate specific proteins. This finding may be a reflection of expression levels of these proteins,

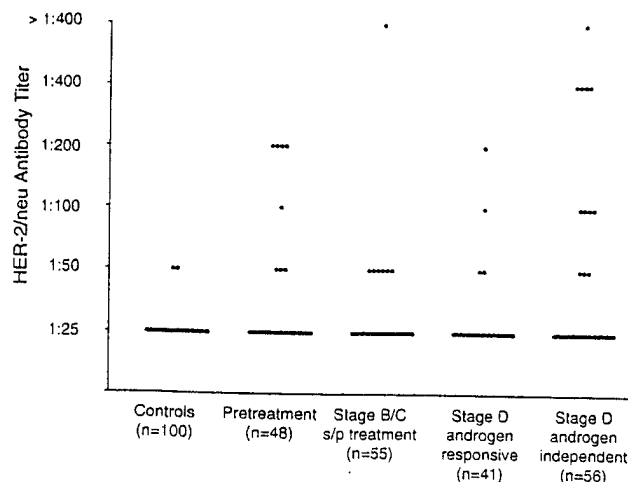


FIG. 5. Antibody immunity to HER-2/neu can be detected in serum of patients with prostate cancer, and is most prevalent in patients with androgen independent disease. Sera from 200 patients with various stages of prostate cancer and 100 male controls were screened for presence of antibodies to HER-2/neu by capture ELISA. Dots represent titers determined for all patients and control blood donors. s/p, status post.

with typically higher serum levels of PSA than PAP detected in patients with metastatic disease, and consequently perhaps greater uptake and antigen presentation. On the other hand, this finding may reflect the inherent humoral immunogenicity of these specific proteins, with perhaps higher tolerance to PAP, which may share epitopes with other tissue phosphatases than PSA. In any case the antibody responses detected were generally low titer, although a few patients had high titer antibodies to PSA or HER-2/neu. The presence of antigen specific immunity detected only in patients with cancer suggests that it is the presence of tumor itself that elicits an immune response. Moreover, the fact that the responses to HER-2/neu were IgG indicates the likely presence of antigen specific CD4⁺ T cell responses mediating immunoglobulin class switch and participating in this immune response.

There was a high prevalence of humoral immune responses to the proteins tested in patients with prostate cancer at titers 1:50 or greater. Of the patients 26% had detectable antibodies to PSA and/or HER-2/neu compared to only 5% of the control population. The prevalence of HER-2/neu-specific antibodies with titers 1:100 or greater, particularly in patients with androgen independent prostate cancer (16%), was similar to what has previously been reported in other adenocarcinomas, such as early stage breast cancer (11%)¹⁸ and colorectal carcinoma (14%).²¹ In contrast, autoantibodies to p53 have been detected in 24% to 30% of patients with ovarian, breast and lung cancers.^{17,19,22} Our results are consistent with studies by Lang et al, who reported a low incidence (3%) of p53 autoantibodies in prostate adenocarcinomas compared with other urogenital tumors.²³ This low prevalence of p53 autoantibodies may be a result of less homogeneous, nuclear accumulation of p53 in prostate cancer compared with other adenocarcinomas.²⁴

In general, we found that the majority of humoral immune responses occurred in patients with androgen independent prostate cancer. The observation that the majority of responses were in patients with late stage disease may reflect that these low level immune responses occur as a result of shed antigens in more bulky disease. Despite the mechanism, however, this observation suggests that even patients with metastatic disease are able to mount an immune response to the tumor.

Tumor antigen specific cytotoxic T lymphocytes have long

been considered critical final effectors in an effective antitumor immune response.²⁵ Others and we have found in a rodent model that a PAP specific Th1 phenotype with PAP specific cytotoxic T lymphocyte is critical for actual destruction of prostate tissue.^{26,27} PSA specific cytotoxic T lymphocyte responses have previously been detected in a patient with prostate cancer, suggesting that a potentially therapeutic immune response can exist in vivo.²⁸ Th1-like immune responses with IgG2 subtype antibody responses are often associated with a concurrent cytotoxic T lymphocyte response. Studies are currently under way to determine the isotype of the IgG prostate antigen specific antibody responses.

CONCLUSIONS

Our findings suggest that prostate cancer is an immunogenic tumor, that is patients with prostate cancer can have antibody immunity to 1 or more proteins associated with the cancer. For PSA and HER-2/neu the prevalence of antibody immunity was higher in patients with end stage disease, suggesting that those with metastatic prostate cancer can have an immune response to the disease. Presently it is unknown whether there are other prostate cancer associated antigens that may be more widely immunogenic but our findings suggest that techniques that have been used in the study of melanoma and renal cell carcinoma to identify antigens recognized by the host immune system may be useful in prostate cancer to define more rational targets for immune based therapies.

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surface protein. Using the phage display combinatorial antibody library and panning over MAT-Ly Lu, we were able to identify distinct set of phages that reacted with cell-specific surface proteins but did not react with purified gp96-peptide complexes. We conclude that the single chain phage display antibody library is a useful reagent to identify intra-cellular and cancer associated cell surface markers and tumor rejection peptides associated with chaperone proteins, gp96. (Funded by the US ARMY Grant # 17-98-1-8534)

#4442 HUMAN ENDOGENOUS RETROVIRUS, HERV-K 10, IS EXPRESSED IN HUMAN BREAST CANCER. Feng Wang-Johanning, M B Khazeali, Theresa V Strong, William E Grizzle, and Albert F LoBuglio, *Univ of Alabama at Birmingham, Birmingham, AL*

As part of an ongoing search for novel tumor antigens, we have analyzed the expression of human endogenous retrovirus (HERV) genes in human cancer tissues. We examined mRNA expression of ERV3, HERV-E 4-1, and HERV-K 10 in breast tissues by reverse transcription-polymerase chain reaction (RT-PCR) and RNA *in situ* hybridization. HERV-K 10 was expressed in most human breast cancer cell lines and most frozen stored samples of breast adenocarcinoma, but not in normal human mammary epithelial cells and tissues. Expression of HERV-K 10 env mRNA was higher in breast tissue containing a higher proportion of tumor cells and in breast cancer cell lines stimulated with female hormones. The expression of HERV-K 10 env mRNA in breast adenocarcinoma was confirmed by RNA *in situ* hybridization using an HERV-K 10 env specific antisense probe. Several RT-PCR fragments of HERV-K 10 from breast cancer tissues were cloned and sequenced. The highest alignment scores for most sequenced clones were with the previously reported HERV-K 10 env gene sequence. Some cloned fragments, which were subcloned into a prokaryotic expression vector, produced stable full-length HERV-K 10 env proteins. We derived polyclonal antibody directed against HERV-K10 env protein, and used the antibody to demonstrate HERV-K 10 env protein expression in tumor epithelial cells. Thus, HERV-K-like transcripts capable of producing stable proteins are commonly expressed in breast cancer. The expression of HERV-K 10 env mRNA transcripts and protein in malignant but not normal breast tissues may provide a tumor-associated antigen for diagnostic and therapeutic applications.

#4443 IMMUNE RESPONSES OF BREAST CANCER PATIENTS TO MUTATED EPIDERMAL GROWTH FACTOR RECEPTOR (MEGF-R). Enkhtsetseg Purev, D. W. Cai, E. Miller, B. Birebent, R. Somasundaram, T. Mayer, and D. Herlyn, *Albert Einstein Med Ctr, Philadelphia, PA, Memorial Hosp of Burlington, Mt. Holly, NJ, and The Wistar Institute, Philadelphia, PA*

mEGF-R is expressed by carcinomas of the breast, ovary, lung and colon, and also by gliomas, but not by normal tissues. The mEGF-R is the result of an 801 bp deletion within the extracellular domain of normal EGF-R and is expressed both on the surface and in the cytoplasm of the tumor cells. Thus, mEGF-R is a potential tumor-specific target for B and/or T cells in active and passive immunotherapy against tumors. We have evaluated humoral and cellular immune responses to mEGF-R in eight breast cancer patients and three healthy donors. Four patients with tumors expressing mEGF-R developed mEGF-R-specific humoral immune responses and two of these patients also developed mEGF-R-specific cellular immune responses. None of the patients with mEGF-R negative tumors developed cellular immune responses, and only one patient developed antibodies to mEGF-R. None of the three healthy donors demonstrated mEGF-R-specific humoral or cellular immune responses. These studies demonstrate that breast cancer patients can immunologically recognize mEGF-R and suggest that enhancement of the immune responses may be possible by vaccination of the patients against mEGF-R. (Supported by DAMD17-96-1-62 37 from the U.S. Department of Defense.)

#4444 IDENTIFICATION OF POTENTIAL CD4+ T HELPER EPITOPES DERIVED FROM THE PROTEIN SEQUENCE OF PROSTATIC ACID PHOSPHATASE (PAP). Douglas G McNeel, Lan D' Nguyen, and Mary L Disis, *Univ of Washington, Seattle, WA*

PAP is a tumor antigen in prostate cancer. Vaccine studies in rodent models indicate cytotoxic T cells (CTL) are critical for generating destructive prostatitis. Consequently, vaccine trials targeting PAP, planned or in progress, are using strategies to maximize the induction of PAP-specific CTL. Several HLA-A2 peptides derived from PAP have already been identified. Immunization with HLA-A2 peptides alone may not be sufficient; the generation of long-lasting CTL responses may require T cell help. Investigations in a variety of models suggest that the addition of T help specific for the immunizing antigen may be effective in generating a robust antigen-specific CTL response. The purpose of this study was to identify potential T helper epitopes derived from PAP for inclusion in peptide-based prostate cancer vaccines. Standard algorithms were used to scan the PAP amino acid sequence for peptides likely to bind human MHC class II molecules. Ten 15- to 18-mer oligopeptides were chosen, constructed, and used as stimulator antigens in proliferative T cell assays using peripheral blood mononuclear cells derived from patients with (n=7) or without (n=13) previously identified T cell responses to PAP protein. Only rare peptide-specific T cell responses were detected in patients with no preexistent PAP protein response. Three peptides tested, however, generated responses in patients with preexistent PAP-specific T cell immunity significantly different from the PAP non-immune population. One of the peptides elicited an immune response in 6/7 PAP-immune

patients tested and none of the PAP non-immune patients. This peptide may represent a universal T helper epitope for inclusion in PAP specific peptide-based vaccines.

#4445 IDENTIFICATION OF TUMOR ENDOTHELIAL CELL-ASSOCIATED ANTIGENS WITH PHAGE DISPLAY TECHNOLOGY. H. J. Bloemendal, A. van Wolfswinkel, M F B G Gebbink, T. Logtenberg, and E. E Voest, *Univ Med Ctr and Utrecht Biotech Systems, Utrecht, Netherlands*

Angiogenesis is a critical step in the progression of tumors from dormancy to a clinically relevant cancer. Inhibition of angiogenesis and vascular targeting are considered new promising anti-cancer therapies. In order to inhibit neovascularization or target the tumor's vasculature it is pivotal to find antigens which are exclusively present on tumor endothelial cells (TECs) and not on endothelial cells of the normal vasculature. We have employed a large phage antibody display library of human single chain Fv (scFv) fragments to isolate scFv against freshly-isolated TECs. The phage library was incubated with single cell suspensions prepared from tumors of patients with renal cell carcinoma and TECs and attached phages were subsequently isolated by cell sorting using the endothelial cell marker Ulex europaeus agglutinin I. After four selection rounds, monoclonal single chain antibody fragments (scFv) were isolated and used in immunohistochemical and flow cytometric analysis. These studies yielded a scFv that recognizes the tumor vasculature in breast, lung and renal carcinoma, whereas adjacent, normal tissues were negative. In flow cytometric analysis, this scFv bound to freshly-isolated TECs but not to cultured human umbilical vein or dermal microvascular endothelial cells. In addition to the TEC's, the scFv only recognized peripheral human B-lymphocytes. Characterization of the epitope recognized by the scFv is in progress. Our study shows that phage display technology is a powerful tool to discover epitopes on TECs that may be associated with novel molecules. These scFv may be used to identify the gene encoding the target molecule and re-formatted to intact human monoclonal antibodies for targeting the tumor vasculature.

#4446 TARGET CELL KILLING BY GENETICALLY ENGINEERED PRIMARY T-CELLS EXPRESSING AN ANTIGEN-SPECIFIC CHIMERIC RECEPTOR. Peter Bernhard Dall, Bettina Durst, Gerd Bauerschmitz, Dieter Niederacher, and Hans G Bender, *MolGenLab, Ob/Gyn, Univ Med Ctr, Duesseldorf, Germany, Ob/Gyn, Duesseldorf, Germany, and Ob/Gyn, Univ Med Ctr, Duesseldorf, Germany*

Different types of cancer cells preferentially express variant epitopes of the CD44v-hyaluronate receptor family on the cell surface. In former studies it could be demonstrated that transfer of a gene encoding the TCR- ζ -chain and a CD44v-specific single-chain-antibody (scFv) into a cytotoxic T-cell line leads to antigen-specific killing of CD44v-expressing target cells. The following questions were: 1. gene transfer efficiency of primary lymphocytes, 2. receptor surface-expression and 3. CD44v-mediated killing. To evaluate the transduction efficiency immediately after the gene transfer a gene coding for a myc-tag was inserted between anti-CD44v7/8-scFv-gene and CD8 α -spacer-gene. The complete construct included genes for the variable domains of heavy and light chain of the CD44v7/8-specific antibody, for the myc-tag, for the CD8 α -spacer and for the TCR- ζ -chain as T-cell-activation domain. This gene was introduced into a retroviral pLXSN-vector. T-cells isolated from the mouse spleen were cocultivated with the retrovirus-producing packaging line Ω E. Gene transfer rates of >9% have been reached. Anti-myc-FACS-analysis of the infected T-cell pool documented surface expression of the chimeric receptor. Cytotoxicity data show killing of CD44v7/8-expressing target cells of up to 45%, depending on the effector/target-ratio. These data show that retargeting of primary T-cells towards a defined antigen induces antigen-specific T-cell cytotoxicity.

#4447 MODIFICATION OF THE TUMOR ANTIGEN GP2 IMPROVES INDUCTION OF GP2-REACTIVE CYTOTOXIC T LYMPHOCYTES. Yoshiyuki Tanaka, Peter S Goedegebuure, and Timothy J Eberlein, *Washington Univ Sch of Medicine, St. Louis, MO*

GP2 (IIISAVVGIL), the p654-662 HER2/neu derived tumor antigen, induces HLA-A2 restricted cytotoxic T lymphocyte (CTL) reactive to various epithelial cancers. However, the binding affinity of GP2 to HLA-A2 has been known as very low. To improve the immunogenicity of GP2, we introduced ten different amino acid substitutions into GP2 at anchor positions. Four out of ten modifications, especially phenylalanine at position 1 (1F) based modifications, showed significant improvement of their binding affinity, which was almost equal to modified gp100 (G9-209 2M), using the T2 stabilization assay. These peptides were used to stimulate peripheral blood lymphocytes from HLA-A2 healthy donors using peptide-pulsed autologous dendritic cells (DC). After 3 times weekly stimulations, CTL activity against GP2 pulsed T2 (T2GP2) and SKOVA2 (SKOV3 HLA-A2 transfectant) was measured in ^{51}Cr release assays. Several modifications significantly enhanced GP2-specific CTL activity. In particular, Phenylalanine at position 1 and Leucine at position 2 (1F2L) based modifications maximized the CTL activity against both T2GP2 and SKOVA2 (about 2.7 fold increase at E/T 40 against SKOVA2). IF2L based modifications increased not only the binding affinity to HLA-A2 but also improved immunogenicity of GP2. These data suggest that DC + modified GP2 may improve immune therapies for the treatment of HER2/neu overexpressing tumors.

(cancer/testis protein of 11 kD), a novel cDNA from the metastatic subline-1F6m. The less aggressive cell line 1F6 showed no expression. Northern blot on a broader panel of human melanoma cell lines with known metastatic capacity after subcutaneous injection into nude mice showed expression of CTP11 only in the highly metastatic cell lines. The full length cDNA contains an ORF coding for a 97 amino acid putative nuclear protein of about 11 kD. Based on the bipartite nuclear localization signal and a C-terminal acidic region this protein could be involved in transcriptional regulation. The gene coding for CTP11 is localized on chromosome Xq26.3-Xq27.1 based on homology with genomic clones. RT-PCR on normal human tissue samples only revealed expression in testis. In human tumor cell lines, highest expression was seen in melanoma and bladder derived cell lines (30%). Nested PCR on fresh human melanocytic lesions demonstrated expression in 7 out of 10 melanomas. No expression was seen in benign melanocytic tumors. Also we found CTP11 expression in some malignant tumors of other histological types. Future studies will include comparisons of expression between CTP11 and other cancer/testis antigens (MAGE, BAGE, GAGE, SSX, NY-ESO1, SCP1,...) and preparation of suitable CTP11 antibodies.

#5589 THE EXPRESSIONS AND THEIR CLINICAL IMPLICATIONS OF CANCER/TESTIS ANTIGENS IN UTERINE CERVICAL CARCINOMA.

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Backgrounds: Cancer/testis antigen (CTAs) are encoded by genes that are silent in virtually all normal adult tissues but expressed in a number of tumors of various histologic types. These gene products are presented by HLA I molecule and recognized by autologous cytolytic T lymphocytes. CTAs includes MAGE, GAGE, and BAGE genes. There is still little information on the expression and their function of these genes in the uterine cervical cancer. Purposes: This study attempted to evaluate the prevalence of cancer/testis antigens and their clinical implication and discuss the possibility of using these results for immunotherapy in the cervical carcinoma. Material and Methods: Thirty-seven cases of primary cervical carcinoma having the data of Human Papilloma Virus -16,18 infection, FIGO staging, and lymph node involvement were studied by RT - nested PCR for MAGE, GAGE, and BAGE genes. Concurrently, these specimens were analysed for DNA sequences after subcloning for identification of PCR products. Additional material from the same specimen was analyzed by in situ- RT PCR using same primers. Results: No expressions of MAGE, BAGE, and GAGE were observed in normal tissues. Expression of BAGE gene was not detected (0.0%). Eleven out of 37 cases expressed MAGE-2 (29.7%) mRNA, 13 out of 37 cases (35.1%) expressed mRNA of GAGE-2. No significant relationships among these gene expressions and invasiveness, lymph node metastasis, and HPV infections were found. Two unknown subtypes of MAGE were identified. Conclusion: The uterine cervical carcinoma could be the targets of immunotherapy using MAGE and GAGE genes, especially GAGE genes may deserve to be considered as a target of immunotherapy in uterine cervical cancer of Korean women.

#5590 CHARACTERIZATION OF TUMOR ANTIGENS RECOGNIZED BY IGG ANTIBODY IN PATIENTS WITH GLIOMA USING SEREX.

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Serological analysis of cDNA expression libraries, SEREX, has recently been proven to be a powerful method for defining immunogenic human tumor antigens. To identify human glioma antigens, we have screened a cDNA library made from two human glioma cell lines, U87MG and T98G, by SEREX with allogeneic sera from 10 glioma patients. A total of 62 positive clones containing 36 distinct cDNAs encoding antigens were isolated. Sequencing analysis showed that 10 cDNA clones encoded previously uncharacterized molecules and 26 represented known gene products, including growth factors, transcription factors, and cell cycle related molecules. We evaluated recognition of these antigens by sera of glioma patients, other brain disease patients, and normal individuals. Among 17 distinct antigens screened, 10 were recognized exclusively by sera from glioma patients, 3 were recognized by sera from both normal and glioma patients, and remaining 4 were recognized by sera from all three groups. Among 10 antigens reacted with only sera from glioma, 3 were reacted with sera from 2 glioma patients or more. These results suggest that proteins expressed in human glioma could be recognized by the immune system in the patients and these antigens may be utilized for diagnosis and treatment of glioma.

#5591 DECAY ACCELERATING FACTOR (CD55) IS OVEREXPRESSED BY COLORECTAL AND GASTRIC CARCINOMAS AND IS CAPABLE OF SIGNAL TRANSDUCTION RESULTING IN PHOSPHORYLATION OF INTRACELLULAR KINASES AND MOBILISATION OF CALCIUM.

Ian Spendlove, and Lindy G Durrant, *Nottingham Univ, Nottingham, United Kingdom*
Decay accelerating factor (CD55) is expressed at low levels by leukocytes, epithelial and endothelial cells. Its normal function is to protect cells from bystander complement activation during an ongoing inflammatory reaction. We have used a panel of antibodies to CD55 to measure the levels of expression on a number of colorectal cell lines by FACS. This revealed an elevation of CD55 expression between 2 and 200 times that seen on normal cells. This result was

confirmed when analysing a panel of 18 freshly dis-aggregated colorectal tumours. Further analysis of tumour sections for CD55 expression revealed large amounts present in the tumour stroma as well as on the epithelial tumour cells. We can also demonstrate that CD55 is deposited into the extracellular matrix of cultured tumour cells. We analysed other potential functions of this overexpressed antigen based on previous observations where CD55 has been shown to have signalling properties in suboptimally stimulated T cells when it is crosslinked using monoclonal antibodies. We have tested this property in CD55 expressing tumour cells by measuring mobilisation of intracellular calcium and activation of the Src kinase pathway. Our preliminary results suggest that using a single antibody to CD55 signalling occurs in tumour cells resulting in Ca^{2+} mobilisation and phosphorylation of intracellular proteins.

#5592 ANTI-IDIOTYPE VACCINE APPROACH TO EGFRVIII-EXPRESSING TUMORS.

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The highest affinity, biologically active Mabs specific for the glioma associated EGFRVIII antigen are generated following combination immunization protocols using synthetic peptide (Pep-3) representing the unique antigenic sequence, and EGFRVIII on cells or membranes, implying the important contribution of tertiary conformation to recognition of this epitope. The murine IgG_{2a} anti-EGFRVIII Mab Y10 (Ab1) was used to generate Lou/M rat anti-idiotypic Mabs (Ab2) following conventional hybridoma protocols. 7 rat Mabs which bound specifically to Y10 F(ab')₂ and inhibited binding of Y10 to Pep-3 by 70-99%, but not the binding of 3 IgG₁ anti-EGFRVIII Mabs (P14, H10, or L8A4) were studied further. 6/7 Ab2 Mabs inhibited the binding of Y10, but not of L8A4, to EGFRVIII+ B16 murine melanoma cells transfected to express murine EGFRVIII (B16msEGFRVIII) by 84-100%. C57Bl/6 mice received 6 immunizations with Ab2 prior to challenge with syngeneic B16msEGFRVIII tumor cells; Ab2 2C7 (IgG_{2a}) immunization resulted in significantly (p<.001) delayed growth, tumor regression in 3/20, and tumor-free status in 6/20 mice. A second Ab2, 5G8, also significantly delayed tumor growth (p<.001), with 3/10 tumor regressors and 1 tumor-free recipient. Both Ab2 2C7 and 5G8 induced Ab1' antibodies specific for EGFRVIII+ cells in rabbits and mice; these Ab1' bound directly to EGFRVIII+ cells and inhibited the binding of Ab1 Y10 to EGFRVIII. These results indicate that EGFRVIII-expressing tumors are targetable by anti-idiotypic vaccines, which have proven to be of clinical value in human trials.

#5593 T CELLS DERIVED FROM PATIENTS WITH PROSTATE CANCER SECRETE IFN γ IN RESPONSE TO STIMULATION WITH PROSTATE CANCER ANTIGENS.

Douglas G McNeel, Lan D Nguyen, and Mary L Disis, *Univ of Washington, Seattle, WA*

Investigations in rodent models suggest that a cytotoxic T cell (CTL) response is required to induce destructive prostatitis, a goal of prostate cancer vaccines. Endogenous immunity against two prostate cancer tumor antigens, prostate specific antigen (PSA) and prostatic acid phosphatase (PAP), has been reported in patients with prostate cancer. In the present study we questioned whether patients with prostate cancer have a measurable T cell response directed against either of these antigens and, if so, whether that response is predominantly Th1 or Th2. Peripheral blood mononuclear cells were obtained from 80 patients with various stages of disease as well as 20 male controls and evaluated for prostate antigen-specific T cell immunity by T cell proliferation in response to antigen stimulation. Significant stimulation index (SI) to PSA, defined as ≥ 2.0 , could be measured in 7.5% (6/80) of prostate cancer patients. PSA-specific T cell immunity was most marked in patients with metastatic disease (15%, 6/40) when compared with controls (0%, 0/20, p=0.01). PAP-specific SI ≥ 2.0 could be detected in 14% of patients (11/80) but was not significantly different from the control population (5%, 1/20, p=0.17). PAP-specific T cell immunity was found in all stages of disease. In general, the majority of T cell responses were of low magnitude (SI < 4). PAP and PSA specific T cells were analyzed for secretion of IFN γ , a measure of a Th1 response, or IL-5, a measure of a Th2 response. The prostate antigen-specific T cells preferentially produced IFN γ . These findings suggest that an appropriate cytokine environment already exists for the generation of CTL directed against prostate cancer antigens and may benefit from augmentation via vaccination.

#5594 INDUCTION OF HLA-A24-RESTRICTED CYTOTOXIC T LYMPHOCYTES IN VITRO BY CYCLOPHILIN B PEPTIDES FROM PATIENTS WITH LUNG CANCER.

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Cyclophilin B (Cyp-B), a family of cyclophilins involved in T cell activation, are identified as a tumor antigen by CTL established from TIL in lung adenocarcinoma. In this study, Cyp-B peptides at positions 91-99 was used for HLA-A24-restricted CTL induction. PBMC (1x10⁶/ml) was cultured with peptides (10 μ M) in the presence of IL-2 (100 IU/ml). PBMC was then restimulated with the irradiated (50Gy) monocytes preincubated with peptides for 2h at day 7 and 14. Cytotoxicity against A24+ lung adenocarcinoma 11-18 cells was examined by 6h ⁵¹Cr-releasing assay after 21 days. High cytotoxicities (over 30% at E/T of 40) were observed in all four HLA-A24+ healthy donors but not a A24- donor. As a second step, PBMC from three A24+ patients with advanced lung adenocarcinoma (stage IIIB/IV) was examined. Induced CTLs from patients were HLA-A24-re-